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Free-Standing Review

α_2-ADRENOCEPTORS AND I_2 SITES IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

NICOLA FRENCH*

Department of Physiology and Pharmacology, University of Strathclyde, Royal College,
204 George Street, Glasgow G1 1XW, Scotland, UK

Abstract—The presence of α_2-adrenoceptors and I_2 sites in the central nervous system is well established. This review provides a complete summary of this literature, commencing with the first description of α_2-adrenoceptors and I_2 sites in the brain and covering in detail their subclassification, distribution, and function. Furthermore, it highlights some important differences between central α_2-adrenoceptors and I_2 sites.

Keywords—α_2-Adrenoceptors, I_2 sites, idazoxan, central nervous system, distribution, function.

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1. INTRODUCTION

It is now clear that a number of the commonly used α_2-adrenoceptor ligands, including clonidine, *p*-aminoclonidine, and idazoxan, also bind with high affinity to a population of nonadrenoceptor sites (Michel and Insel, 1989). To distinguish them from α_2-adrenoceptors, these have been termed imidazoline (I) sites. This reflects the fact that many of the ligands that bind to these sites possess an imidazoline(-like) moiety, although it is now recognised that this structural peculiarity is neither necessary nor sufficient to allow high-affinity binding to I sites.

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*Present address: Medical Affairs Department, Knoll Pharmaceuticals Ltd., 9 Castle Quay, Castle Boulevard, Nottingham NG7 1FW, UK.

Abbreviations—ACh, acetylcholine; CDS, clonidine-displacing substance; GFAP, glial fibrillary acidic protein; 5-HT, 5-hydroxytryptamine; LCGU, local cerebral glucose use; MAO, monoamine oxidase; NA, noradrenaline.

The classification of I sites into I_1 and I_2 subtypes has been proposed on the basis of results obtained from radioligand-binding studies (Michel and Insel, 1989; Michel and Ernsberger, 1992). Broadly speaking, I_1 sites are defined according to their high (nanomolar) affinity for clonidine and *p*-aminoclonidine (Bricca *et al.*, 1989; Ernsberger *et al.*, 1990), and I_2 sites, according to their high (nanomolar) affinity for idazoxan (Wikberg, 1989; Zonnenschein *et al.*, 1990). It should be noted, however, that idazoxan also has a relatively high affinity for I_1 sites (Bricca *et al.*, 1989; Ernsberger *et al.*, 1990).

This review describes in detail the properties, distribution, and function of α_2-adrenoceptors and I_2 sites in the mammalian brain. In addition, it aims to demonstrate that despite some similarities in their ligand recognition properties, central α_2-adrenoceptors and I_2 sites are distinct and, as such, function as discrete entities in the brain. Although α_2-adrenoceptors and I_2 sites are also localised throughout the periphery, this will receive little attention unless it is pertinent to studies of the CNS. Similarly, the focus on I_1 sites, which are present both peripherally and centrally, will be minimal.

2. α_2-ADRENOCEPTORS: A HISTORICAL PERSPECTIVE

The term *adrenoceptor* refers to the membrane-bound receptor responsible for mediating the effects of endogenous adrenaline and noradrenaline (NA) in the periphery and CNS. The division of adrenoceptors into α and β subtypes was prompted by Ahlquist in 1948, following the demonstration that the effects of a range of sympathomimetic compounds on various smooth muscle preparations displayed two distinct rank orders of potency. Almost 20 years later, Lands *et al.* (1967) proposed the delineation of β-adrenoceptors into two subtypes, β_1 and β_2. Once again, this proposal was based on differences in the rank order of potency of various sympathomimetics, this time on β-adrenoceptor-mediated responses in the heart and vascular smooth muscle.

The subdivision of α-adrenoceptors originally was made on the basis of anatomical rather than pharmacological evidence. Thus, α-adrenoceptors located on peripheral sympathetic nerve terminals were designated α_2-adrenoceptors to distinguish them from the postsynaptic α_1-adrenoceptor population (Langer, 1974). Subsequent pharmacological data were indeed consistent with the existence of two classes of α-adrenoceptor. Thus, for a range of α-adrenoceptor agonists, the pre- and postsynaptic receptors exhibited distinct selectivity profiles (see Berthelsen and Pettinger, 1977). However, those receptors with the pharmacological characteristics of Langer's "presynaptic" subtype were also demonstrated *postsynaptically* and, as a result, it was suggested that the subclassification of α-adrenoceptors should be made on a pharmacological rather than on an anatomical basis (Berthelsen and Pettinger, 1977). To this end, a large number of compounds exhibiting selectivity for α_1- and α_2-adrenoceptors has been developed. Those used most frequently are listed in Table 1.

2.1. α_2-Adrenoceptor Subtypes

According to current views, α_2-adrenoceptors can be further divided into at least four subtypes. Evidence for this has been generated from two sources: namely, radioligand-binding assays and molecular biological studies. These are discussed in detail below.

2.1.1. *Radioligand-Binding Work*

Proposals for the heterogeneous nature of α_2-adrenoceptors were originally based on differences in the pharmacological characteristics of the receptors between species. Thus, α_2-adrenoceptors in various rodent tissues displayed a slightly lower affinity for yohimbine than α_2-adrenoceptors in nonrodent tissue (Cheung *et al.*, 1982; Feller and Bylund, 1984). In addition, prazosin was able to displace antagonist-binding more efficiently from rodent α_2-adrenoceptors (Cheung *et al.*, 1982; Latifpour *et al.*, 1982; Summers *et al.*, 1983), while oxymetazoline had a much higher affinity for the α_2-adrenoceptors of nonrodent tissue (Feller and Bylund, 1984).

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Table 1. *Pharmacological Agents Commonly Used to Distinguish Between α_1- and α_2-Adrenoceptors*

Adrenoceptor subtype	Agonists	Antagonists	
α_1	phenylephrine	phenoxybenzamine	
	methoxamine	prazosin	
	cirazoline	WB4101[1]	
α_2	clonidine	yohimbine	rauwolscine
	p-aminoclonidine	idazoxan	efaroxan
	UK-14,304[2]	RX811059[3]	RX821002[4]
	oxymetazoline	SKF 104078[5]	

[1] *N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-1,4-benzodioxan-2-methanamine.

[2] 5-bromo-6-[2-imidazolin-2-ylamino]quinoxaline.

[3] 2-(2-ethoxy-1,4-benzodioxan-2-yl)-2-imidazoline.

[4] 2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline.

[5] 6-chloro-9-(3-methyl-2-butenyl)oxyl-3-methyl-1*H*-2,3,4,5-tetrahydro-3-benzazepine maleate.

Subsequent radioligand-binding studies demonstrated that α_2-adrenoceptors displaying differential sensitivity to prazosin and oxymetazoline were present not only within the same species, but also within the same tissue, indicating that the subtypes defined in the earlier studies were not simply species-specific forms of the same receptor (Bylund, 1985; Petrash and Bylund, 1986). These subtypes were termed α_{2A} (high affinity for oxymetazoline) and α_{2B} (high affinity for prazosin). Other compounds that distinguish between α_{2A} and α_{2B} receptors have been identified since, largely with the aid of tissues and cell lines shown to express only one of the two subtypes. These are summarised in Table 2.

These tissues and cell lines have also been exploited for the study of the functional characteristics of the receptors they express. Thus, Bylund and Ray-Prenger (1989) examined the ability of a range of α-adrenoceptor antagonists to attenuate UK-14,304-induced inhibition of cyclic 3′,5′-adenosine monophosphate production in two cell lines, one containing only α_{2A}-adrenoceptors (HT29 cells) and the other containing only α_{2B}-adrenoceptors (NG108 cells). Not only was the rank order of potency for the various antagonists consistent with that predicted from radioligand-binding work, but there was also a highly significant correlation between the ability of the antagonists to attenuate the response to UK-14,304 and their affinity for each subtype. Such findings were to add considerable weight to the proposed subdivision of α_2-adrenoceptors into α_{2A} and α_{2B} subtypes.

Evidence for a third subtype once again was based on results from radioligand-binding studies. Thus, the pharmacological profile of the α_2-adrenoceptor expressed in the opossum kidney-derived cell line was found to be distinct from that of both the α_{2A} and the α_{2B} subtypes. While it had a

Table 2. *Pharmacological Agents Used to Distinguish Between α_{2A}- and α_{2B}-Adrenoceptors*

	Affinity[1] (nM)		Selectivity ratio	
Compound	α_{2A}	α_{2B}	α_{2A}:α_{2B}	Reference
BRL 44408	1.7	144.5	85	Young *et al.*, 1989
Oxymetazoline	7.8	251.2	32	Michel, A. D. *et al.*, 1989
Benoxathian	22.4	426.6	19	Michel, A. D. *et al.*, 1989
BAM 1303	2.5	25.7	10	Simmoneaux *et al.*, 1991
ARC 239	169.8	1.6	0.0094	Simmoneaux *et al.*, 1991
BRL 41992	103.3	1.1	0.011	Young *et al.*, 1989
Prazosin	4677.3	60.2	0.013	Michel, A. D. *et al.*, 1989
Imiloxan	3019.9	55.0	0.018	Michel, A. D. *et al.*, 1989
Spiroxatrine	93.3	3.4	0.036	Michel, A. D. *et al.*, 1989
Chlorpromazine	871.0	52.5	0.06	Michel, A. D. *et al.*, 1989

[1] The affinity of each ligand for the α_{2A}- and α_{2B}-adrenoceptors was determined according to their ability to displace [^{3}H]rauwolscine from tissues or cell lines containing only one of the two subtypes.

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high affinity for prazosin and ARC 239 and a low affinity for oxymetazoline, characteristics indicative of the α_{2B} subtype, its affinity for yohimbine was in the range expected for an interaction at the α_{2A}-adrenoceptor (Murphy and Bylund, 1988). Furthermore, the ratio of the affinities of prazosin and yohimbine (40) was intermediate between that obtained for the α_{2A}- (240–570) and α_{2B}-adrenoceptors (5). Accordingly, the receptor expressed by the opossum kidney cell line was designated the α_{2C} subtype. Subsequent studies have confirmed that despite some similarities in their ligand recognition properties, the α_{2B} and α_{2C} subtypes *are* distinct. Thus, Blaxall *et al.* (1991) have shown that of 49 compounds tested, 5 (rauwolscine, WB4101, BAM 1303, raubasine, and akuammigine) have a significantly (at least 10-fold) higher affinity for the α_2-adrenoceptor in the opossum kidney cell line than for the α_2-adrenoceptor in neonatal rat lung (α_{2B}).

A fourth subtype, α_{2D}, has been identified in rat submaxillary gland (Michel, A. D. *et al.*, 1989) and bovine pineal gland (Simonneaux *et al.*, 1991). It is distinguished from the other three subtypes on the grounds of its unique pharmacological characteristics. Most notable is its low affinity for rauwolscine compared with the α_{2A}, α_{2B}, and α_{2C} subtypes. In addition, the affinity of the α_{2D}-adrenoceptor for prazosin, mianserin, and BAM 1303 differentiate it from at least one of the other three subtypes (Michel, A. D. *et al.*, 1989; Simonneaux *et al.*, 1991; Renouard *et al.*, 1994).

At present, there is no single ligand that clearly discriminates one α_2-adrenoceptor subtype from the other three. For this reason, the assignation of α_2-adrenoceptors to a particular subtype is not straightforward. Typically, it involves a two-step process. First, the affinities of a range of compounds (selected according to their ability to differentiate one α_2-adrenoceptor subtype from at least one of the other three) for the α_2-adrenoceptor in question are determined. Second, these values are correlated with those obtained from tissues/cell lines containing only one of the four α_2-adrenoceptor subtypes. Alternatively, some groups have expressed the genes encoding each α_2-adrenoceptor subtype (see Section 2.1.2) in cell lines, and determined their pharmacological profile in this way. In addition, the observation that the ratio of the affinities for certain pairs of compounds appears to be indicative of specific subtypes has been exploited in the designation of α_2-adrenoceptors to a particular subtype (see, for example, Simonneaux *et al.*, 1991).

2.1.2. *Molecular Biological Studies*

Using molecular biological techniques, three genes encoding human α_2-adrenoceptors have been cloned: α_2-C10 (Kobilka *et al.*, 1987), α_2-C2 (Lomasney *et al.*, 1990; Weinshank *et al.*, 1990), and α_2-C4 (Regan *et al.*, 1988). According to their pharmacological profile, these genes appear to represent, respectively, the α_{2A}, α_{2B}, and α_{2C} subtypes defined in radioligand-binding studies (see Section 2.1.1). Three genes encoding α_2-adrenoceptors have also been identified in rats: α_2-RNG (Zeng *et al.*, 1990), α_2-RG10 (Flordellis *et al.*, 1991; Lanier *et al.*, 1991; Voigt *et al.*, 1991), and α_2-RG20 (Lanier *et al.*, 1991); these appear to correspond, pharmacologically, to the α_{2B}, α_{2C}, and α_{2D} subtypes, respectively (see Zeng and Lynch, 1991).

The proteins encoded by the human α_2-C2 and rodent α_2-RG10 genes seem to represent species homologues of the α_{2B}-adrenoceptor subtype: not only are they pharmacologically very similar, but their amino acid sequences are 82% identical (see Bylund, 1992; Ruffolo *et al.*, 1993). For the same reasons, the human α_2-C4 and the rat α_2-RG10 proteins are considered to be species homologues of the α_{2C}-adrenoceptor (Bylund, 1992; Ruffolo *et al.*, 1993). Since there is a high degree of sequence homology between the human α_2-C10 and the rat α_2-RG20 proteins (89%), it has been proposed that these subtypes also represent species homologues (Harrison *et al.*, 1991). However, the pharmacology of the α_{2A}-adrenoceptor is distinct from that of the α_{2D} subtype (see Michel, A. D. *et al.*, 1989; Simonneaux *et al.*, 1991; Section 2.1.1), indicating that they should be classified separately. Failure to recognise or accept this has led to some confusion concerning the α_2-adrenoceptor subtype (α_{2A} or α_{2D}) under scrutiny in studies using nonhuman (rodent/bovine) tissue. To avoid such confusion, the following nomenclature will be adopted in the remainder of this review: the term Hα_{2A} will be used to denote the α_2-adrenoceptor corresponding to the human α_2-C10 receptor (*i.e.*, that with the pharmacological characteristics of the α_{2A} subtype), while the term Rα_{2A} will be reserved to identify the α_2-adrenoceptor corresponding to the rat α_2-RG20 receptor (*i.e.*, that with the pharmacological characteristics of the α_{2D} subtype; see Millan *et al.*, 1994; Renouard *et al.*, 1994).

Table 3. *α_2-Adrenoceptor Subtypes: Summary of Radioligand-Binding Studies and Molecular Biological Work*

α_2-Adrenoceptor subtype[1]	Genomic clone		
	Human	Rat	Mouse
$H\alpha_{2A}$ (α_{2A})	α_2-C10	–	–
α_{2B}	α_2-C2	α_2-RNG	–
α_{2C}	α_2-C4	α_2-RG10	$M\alpha_2$-4H
$R\alpha_{2A}$ (α_{2D})	–	α_2-RG20	$M\alpha_2$-10H

[1] As defined in radioligand-binding studies.

Two genes encoding murine α_2-adrenoceptors have also been identified ($M\alpha_2$-4H and $M\alpha_2$-10H; Link *et al.*, 1992). In terms of its predicted amino acid sequence and pharmacological characteristics, $M\alpha_2$-4H coincides with the human α_2-C4 receptor and, therefore, appears to represent the α_{2C} subtype. The amino acid sequence of $M\alpha_2$-10H is very similar to that of the human α_2-C10 receptor, while its pharmacology is more akin to that of the rat α_2-RG20 receptor; this suggests that $M\alpha_2$-10H corresponds to the $R\alpha_{2A}$ subtype.

A summary of the results obtained from the radioligand-binding studies and molecular biological work is presented in Table 3.

2.1.3. *Further Subclassification*

More recently, it has been proposed that the $R\alpha_{2A}$- and α_{2B} subtypes from certain rodent tissues can each be subdivided into two types ($R\alpha_{2A1}$ and $R\alpha_{2A2}$; α_{2B1} and α_{2B2}) according to their sensitivity to guanoxabenz (Uhlén *et al.*, 1993; Xia *et al.*, 1993). These claims await further pharmacological and functional evaluation. It is interesting to note, however, that the $R\alpha_{2A2}$ subtype appears to be encoded by the RG20 gene and the α_{2B2} subtype by the RNG gene (Uhlén *et al.*, 1993; Xia *et al.*, 1993).

2.2. α_2-Adrenoceptors in the Brain

In the late 1970s, U'Prichard, Snyder, and their co-workers produced a plethora of papers professing the presence of α-adrenoceptors in rodent and bovine brain (Greenberg *et al.*, 1976; Greenberg and Snyder, 1977; U'Prichard and Snyder, 1977a,b; U'Prichard *et al.*, 1977a,b; Peroutka *et al.*, 1978). The technique used was, of course, radioligand-binding, and the ligands employed were [^{3}H]adrenaline, [^{3}H]NA, [^{3}H]clonidine, [^{3}H]dihydroergocryptine, and [^{3}H]WB4101. Given the timing of the publication of these papers, the criteria applied for the identification of α-adrenoceptors did not take into account the existence of subtypes. In retrospect, it is clear that the receptors labelled by [^{3}H]clonidine (as well as [^{3}H]adrenaline and [^{3}H]NA; see Miach *et al.*, 1978; U'Prichard and Snyder, 1979) represent α_2-adrenoceptors, and as such, this work provided the first categorical evidence for the presence of this receptor in the mammalian CNS.

Subsequent radioligand-binding studies have identified α_2-adrenoceptors in the CNS of a number of other mammalian species, including mouse (Rago *et al.*, 1992; Hussain *et al.*, 1993), guinea pig (Wikberg, 1989; Hussain *et al.*, 1993), rabbit (Convents *et al.*, 1989; Renouard *et al.*, 1993), cat (Howe and Yaksh, 1984; Parkinson *et al.*, 1988), dog (Robertson and Leslie, 1985; Klein *et al.*, 1988), sheep (Herdon *et al.*, 1983), monkey (Rakic *et al.*, 1988; Lidow *et al.*, 1989), and human (Weinreich and Seeman, 1981; Summers *et al.*, 1983; Cash *et al.*, 1985).

There is now good evidence to suggest that each of the four α_2-adrenoceptor subtypes is expressed in mammalian brain tissue. First, pharmacological characterisation of the α_{2A} and α_{2B} subtypes was performed using a variety of tissues, including brain, indicating that these subtypes are present in the CNS (Cheung *et al.*, 1982; Bylund, 1985; Petrash and Bylund, 1986; see Section 2.1.1). It should be noted, however, that the α_2-adrenoceptor identified as the α_{2B} subtype in these early studies appears to correspond to the more recently defined α_{2C} subtype (see Zeng and Lynch, 1991; O'Rourke *et al.*, 1992; McCune *et al.*, 1993; Wallace *et al.*, 1994). Second, the genes encoding the α_{2C} (Flordellis *et al.*, 1991; Lanier *et al.*, 1991; Voigt *et al.*, 1991) and $R\alpha_{2A}$ (Lanier *et al.*,

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1991) subtypes were cloned from rat brain, supporting a central localisation of these receptors (see Section 2.1.2).

Using Northern blot analysis and *in situ* hybridisation, it has been shown that the mRNA corresponding to the $R\alpha_{2A}$, α_{2B}, and α_{2C} subtypes is present in rat brain (Zeng and Lynch, 1991; McCune *et al.*, 1993; Nicholas *et al.*, 1993). Further evidence that the $R\alpha_{2A}$ subtype is present in the rat brain has been obtained by two independent groups. Both MacKinnon *et al.* (1992a) and Heal *et al.* (1994) have shown that α_2-adrenoceptors in the rat cerebral cortex have a similar pharmacological profile to the prototypical $R\alpha_{2A}$ (α_{2D})-adrenoceptors expressed in rat submaxillary gland. In human brain, the presence of mRNA encoding the $H\alpha_{2A}$- and α_{2C}-adrenoceptor subtypes has also been established (Perälä *et al.*, 1992).

Finally, Uhlén *et al.* (1993) have proposed that the recently described $R\alpha_{2A2}$ subtype is localised in rat cerebral cortex, while the $R\alpha_{2A1}$ subtype is absent. The presence of the putative α_{2B} subtypes in the brain has yet to be examined.

2.2.1. *Localisation*

The availability of a number of α_2-adrenoceptor–selective ligands in radiolabelled form means that the localisation of central α_2-adrenoceptors has been examined in detail using receptor autoradiography.

In rat brain, the distribution of α_2-adrenoceptors has been assessed using $[^3H]$*p*-aminoclonidine (Young and Kuhar; 1980; Unnerstall *et al.*, 1984), $[^3H]$rauwolscine (Boyajian *et al.*, 1987) and $[^3H]$idazoxan (Boyajian *et al.*, 1987; Brüning *et al.*, 1987). However, the use of each of these compounds is compromised by their additional interaction at nonadrenoceptor sites in rat brain. Thus, *p*-aminoclonidine and idazoxan bind to I sites (Bricca *et al.*, 1989; Wikberg, 1989; Brown *et al.*, 1990; Ernsberger *et al.*, 1990; see Section 3) and rauwolscine binds to the 1A subtype of 5-hydroxytryptamine (5-HT) receptors (Broadhurst and Wyllie, 1986).

For this reason, Hudson *et al.* (1992b) have used the tritiated form of RX821002 (see Table 1) to determine the autoradiographic localisation of α_2-adrenoceptors in the rat brain. This ligand, which is the 2-methoxy analogue of idazoxan (Stillings *et al.*, 1985), is a highly selective α_2-adrenoceptor antagonist that lacks the additional interaction at nonadrenoceptor I sites in rat (Mallard *et al.*, 1992; De Vos *et al.*, 1994), as well as human (Miralles *et al.*, 1993a) brain. The results obtained, which are summarised in Sections 2.2.1.1–2.2.1.6, demonstrate the widespread and heterogeneous distribution of α_2-adrenoceptors in the rat brain.

2.2.1.1. *Cerebral cortex.* The frontal, cingulate, parietal, and occipital cortices all contain moderate densities of α_2-adrenoceptors. In areas 1 and 2 of the frontal cortex, the density is particularly high.

2.2.1.2. *Telencephalon.* The lateral septum contains the highest density of α_2-adrenoceptors in the rat brain. High levels of binding are also present throughout the anterior olfactory nucleus, in the bed nucleus of the stria terminalis, in the ventral pallidum and, to a lesser extent, in the nucleus accumbens and stria terminalis. In contrast, the globus pallidus and caudate putamen exhibit low levels of binding.

The distribution of α_2-adrenoceptors in the hippocampal formation is very heterogeneous. Thus, the dentate gyrus and hippocampal fissure are very densely labelled, while the subiculum and the CA fields of the hippocampus proper are labelled with moderate intensity. In addition, the entorhinal cortex contains a high level of α_2-adrenoceptors.

2.2.1.3. *Diencephalon.* In the thalamus, a particularly high density of α_2-adrenoceptors is associated with the rhomboid, ventrolateral laterodorsal, and central medial thalamic nuclei. Lower levels are present in the dorsomedial anteroventral, paraventricular, and reticular nuclei. In the medial geniculate nuclei, binding is heterogenous, with high levels in the dorsal area and much lower levels in the ventral regions.

In the hypothalamus, the α_2-adrenoceptor density is moderate in the medial mammillary nucleus and progressively higher in the arcuate nucleus, ventromedial hypothalamic nucleus, and lateral hypothalamic area.

Finally, the amygdala contains generally high levels of α_2-adrenoceptors, particularly in the basomedial and basolateral nuclei.

2.2.1.4. *Mesencephalon.* The inferior colliculus displays a very high density of α_2-adrenoceptors, particularly in dorsal areas of the cortex. In the superior colliculus, the distribution of α_2-adrenoceptors is uneven, with moderate levels in the superficial gray layer and lower levels in the ventral gray layers. Moderate levels are also found in the central gray, the interpeduncular nucleus, and the zona compacta of the substantia nigra, while low levels occur in the reticular area of the substantia nigra and the habenular nuclei.

2.2.1.5. *Rhombencephalon.* The density of α_2-adrenoceptors in the cerebellum is generally low, although the granule cell layer contains twice as many receptors as the molecular layer. In the brainstem and pons, binding is markedly elevated in the nucleus parabrachialis lateralis and locus coeruleus. More caudally, a high density of α_2-adrenoceptors is present in the nucleus tractus solitarius and the spinal trigeminal nucleus. In contrast, the area postrema contains a low number of α_2-adrenoceptors.

2.2.1.6. *Spinal cord.* In the dorsal horn of the spinal cord, a high density of α_2-adrenoceptors is present in the superficial layers (laminae 1–3). The other layers contain moderate levels.

[^{3}H]RX821002 has also been used to determine the autoradiographic localisation of α_2-adrenoceptors in human brain (De Vos *et al.*, 1994). The most striking feature of the results obtained is that in comparison with the rat brain (Hudson *et al.*, 1992b), many regions of the human brain contain a much lower density of α_2-adrenoceptors. Other notable differences include the apparent lack of binding in the dentate gyrus and the homogeneously low levels of receptors in the thalamus and brainstem.

These results must be regarded with a suitable degree of caution, however, since they differ considerably from those published by Pascual *et al.* (1992). Using the α_2-adrenoceptor agonist, [^{3}H]UK-14,304, this group demonstrated that the density of α_2-adrenoceptors in many of the regions in human brain is well in excess of that reported for the same regions in rat brain (Hudson *et al.*, 1992b). Although UK-14,304 has an imidazoline-like structure, the possibility that the results obtained are compromised by an interaction of this compound with I sites can be ruled out. Thus, De Vos *et al.* (1994) have shown that UK-14,304 has a relatively low affinity for these sites in human brain (see also Miralles *et al.*, 1993a).

Several groups have reported a regionally specific reduction in α_2-adrenoceptor density in human brain with age (Cash *et al.*, 1987; Pascual *et al.*, 1992; Sastre and García-Sevilla, 1993). It is possible, therefore, that the discrepancies may relate to differences in the ages of the subjects from which the tissue was derived. Indeed, the age range of the 6 subjects used in the study of De Vos *et al.* (1994) was 46–83, while the 22 subjects used by Pascual *et al.* (1992) were aged between 19 and 84, and 7 of these were aged 46 or less. Further studies are required to clarify the situation. In the meantime, it is difficult to draw any firm conclusions concerning the relative densities of α_2-adrenoceptors in discrete regions of the human brain.

Information regarding the autoradiographic localisation of α_2-adrenoceptor subtypes is not available yet. However, several groups have examined the distribution of mRNA corresponding to the various α_2-adrenoceptor subtypes in rat brain using either Northern blot analysis (Zeng and Lynch, 1991) or *in situ* hybridisation (McCune *et al.*, 1993; Nicholas *et al.*, 1993; Scheinin *et al.*, 1994). The results derived from such experiments demonstrate that mRNA encoding each of the three α_2-adrenoceptor subtypes is present in rat brain. However, the localisation of each is distinct. Thus, while the α_{2B} mRNA appears to be restricted to the thalamus, the Rα_{2A} and α_{2C} mRNA is widely and differentially distributed. Those regions that contain Rα_{2A}, but not α_{2C}, mRNA include numerous brainstem nuclei (the locus coeruleus, the A4, A5, and A7 catecholamine cell groups, the nucleus parabrachialis lateralis, and the dorsal motor nucleus of the vagus), the raphe nuclei and the septum. In contrast, the olfactory tubercle and basal ganglia (caudate, nucleus accumbens, and islands of Calleja) express messages for α_{2C}-adrenoceptors only. Rα_{2A} and α_{2C} mRNA is present in the cerebral cortex, hippocampus, thalamus, hypothalamus, and cerebellum (Zeng and Lynch, 1991; McCune *et al.*, 1993; Nicholas *et al.*, 1993; Scheinin *et al.*, 1994).

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For the determination of actual receptor localisation, this approach is less useful than radioligand-binding since the distribution pattern of mRNA represents the cells of *origin* of the receptors rather than their sites of functional accumulation. For example, numerous groups have reported that while $R\alpha_{2A}$ and α_{2C} binding sites are present in the rat cerebral cortex, the $R\alpha_{2A}$ subtype predominates (90:10; Uhlén and Wikberg, 1991; O'Rourke *et al.*, 1992; Wallace *et al.*, 1994). In contrast, roughly equivalent amounts of mRNA for the two subtypes are expressed in this brain region (Zeng and Lynch, 1991; McCune *et al.*, 1993; Nicholas *et al.*, 1993; Scheinin *et al.*, 1994).

The presence of mRNA encoding the $H\alpha_{2A}$- and α_{2C}-adrenoceptor subtypes has also been established in a number of regions of the human brain (Perälä *et al.*, 1992). The results obtained demonstrate that the message for both subtypes occurs in the pons, ventral hypothalamus, and amygdala, while the $H\alpha_{2A}$ mRNA predominates in the cerebral cortex and the α_{2C} mRNA predominates in the caudate. The periventricular thalamus and cerebellar cortex were noted to contain only $H\alpha_{2A}$-specific mRNA. This work has been confirmed and extended by Ordway *et al.* (1993), who determined the α_2-adrenoceptor subtypes present in homogenates from various human brain regions using radioligand-binding techniques. Their results show that the α_2-adrenoceptors localised in the cerebral cortex are mainly of the $H\alpha_{2A}$ subtype, while those in the caudate comprise mainly the α_{2C} subtype. In addition, while both subtypes were reported to be present in the hippocampus, amygdala, mammillary body, cerebellum, and locus coeruleus, $H\alpha_{2A}$-adrenoceptors predominate in these regions. The distribution of the α_{2B} subtype in human brain is unclear, although Ordway *et al.* (1993) have inferred from their results that it may only be present in the amygdala.

2.2.2. *Function*

As discussed in the preceding section, central α_2-adrenoceptors are distributed heterogenously throughout almost the entire neuroaxis. Accordingly, these receptors are thought to mediate a wide range of functions, each of which is detailed below.

2.2.2.1. *Regulation of neurotransmitter release.* Perhaps the most important, or at least the best defined, function of α_2-adrenoceptors in the brain is the regulation of central NA release. Thus, NA released into the synaptic cleft is thought to stimulate presynaptic α_2-adrenoceptors ("autoreceptors"), triggering a negative feedback mechanism that acts to inhibit subsequent neurotransmitter release.

The chain of events leading to the conceptualisation of adrenergic autoreceptors was initiated by Brown and colleagues, who consistently demonstrated that α-adrenoceptor antagonists such as phenoxybenzamine enhanced the stimulation-evoked overflow of [^{3}H]NA from sympathetic neurones in the peripheral nervous system (Brown and Gillespie, 1957; Brown *et al.*, 1958; Brown, 1965). Several theories were advanced to account for this observation, including the reduced ability of NA to combine with postsynaptic α-adrenoceptors in the presence of blocking agents (Brown and Gillespie, 1957; Brown *et al.*, 1958; Brown, 1965) and the inhibition of neuronal (Theonen *et al.*, 1964) and extraneuronal (Eisenfeld *et al.*, 1967) uptake of NA. However, independent studies carried out by Langer (1970) and Starke *et al.* (1971) indicated that such explanations were not entirely satisfactory, and it was proposed that α-adrenoceptor antagonists increased overflow by facilitating neurotransmitter release itself (De Potter *et al.*, 1969; Langer, 1970; Starke *et al.*, 1971).

Since the concentration of phenoxybenzamine shown to increase NA release was within the range required for α-adrenoceptor blockade, it was concluded that the mechanism underlying facilitation of release involved the antagonism of α-adrenoceptors. Although it originally was assumed that these receptors were postsynaptic, the observation that phenoxybenzamine increased NA release from preparations considered *not* to contain postsynaptic α-adrenoceptors (*e.g.*, rabbit heart; Starke *et al.*, 1971) was strong evidence that, in fact, the receptors were located presynaptically. Thus, to explain the facilitatory effects of phenoxybenzamine, it was hypothesised that during neurotransmission, NA was released into the synapse where, in addition to acting on postsynaptic adrenoceptors, it also stimulated *presynaptic* α-adrenoceptors, initiating a negative feedback mechanism that inhibited subsequent NA release. In this way, phenoxybenzamine would be seen to increase NA release by interrupting such autoinhibitory feedback.

The results obtained with phenoxybenzamine subsequently were replicated using a wide range of α-adrenoceptor antagonists in various sympathetically innervated tissues from a number of different species (see Westfall, 1984, for a review). Additional support for the concept of noradrenergic autoinhibition came from studies using α-adrenoceptor *agonists*, which consistently *inhibited* stimulation-evoked NA release (Westfall, 1984). As discussed in Section 2, α-adrenoceptors have been subdivided into two types on the basis of differing pharmacological properties (Berthelson and Pettinger, 1977). In retrospect, the agonists/antagonists shown to depress/facilitate [^{3}H]NA overflow were either nonselective α-adrenoceptor ligands or α_2-selective compounds. Consequently, the presynaptic receptors controlling NA release in the periphery were classified as the α_2 subtype.

It is now clear that release-regulating α_2-adrenoceptors are also present on the terminals of noradrenergic neurones in the CNS. Thus, a number of studies consistently have demonstrated that α_2-adrenoceptor agonists decrease, while antagonists increase, the stimulus-evoked release of [^{3}H]NA from brain slices and synaptosomes of a variety of mammalian species, including rat (Baumann and Koella, 1980; Cichini *et al.*, 1986; Valenta *et al.*, 1988; El Mas and Hughes, 1990), mouse (Schlicker *et al.*, 1994), rabbit (Reichenbacher *et al.*, 1982; Heepe and Starke, 1985; Mayer *et al.*, 1988), and human (Feuerstein *et al.*, 1990; Raiteri *et al.*, 1992). These results have been confirmed *in vivo* using intracerebral microdialysis (L'Heureux *et al.*, 1986; Routledge and Marsden, 1987; Abercrombie *et al.*, 1988; Itoh *et al.*, 1990; Marsden, 1990; Abercrombie and Findlay, 1991; Thomas and Holman, 1991; van Veldhuizen *et al.*, 1993). The observation that α_2-adrenoceptor antagonists increase basal NA levels *in vivo* is particularly interesting since it suggests that NA release in the brain is tonically regulated by α_2-autoreceptors.

In addition to their presynaptic location, α_2-autoreceptors are found on the cell bodies of locus coeruleus neurones (Cedarbaum and Aghajanian, 1977). Stimulation of these "somatodendritic" receptors also results in a depression of NA release in terminal regions, in this case via a reduction in the firing rate of locus coeruleus projection neurones (Svensson *et al.*, 1975).

The results obtained from a substantial number of *in vitro* studies suggest that inhibitory α_2-adrenoceptors (or "heteroreceptors") are also present on the terminals of 5-HT-containing neurones in a number of brain regions in a variety of species (rat: Starke and Montel, 1973; Frankhuyzen and Mulder, 1980; Galzin *et al.*, 1984; guinea pig: Raiteri *et al.*, 1990; rabbit: Feuerstein *et al.*, 1985; Limberger *et al.*, 1986; pig: Fink *et al.*, 1988; human: Raiteri *et al.*, 1990; see Göthert and Schlicker, 1991, for a review). Thus, α_2-adrenoceptor agonists decrease the evoked release of [^{3}H]5HT from preloaded brain slices and/or synaptosomal preparations. Evidence that α_2-heteroreceptors are tonically activated by endogenous NA is equivocal, however. For example, α_2-adrenoceptor antagonists are able to block the inhibitory effect of exogenous NA, but they do not appear to affect the evoked release of [^{3}H]5HT *per se* (Raiteri *et al.*, 1983; Galzin *et al.*, 1984; Limberger *et al.*, 1986; Maura *et al.*, 1992). On the other hand, Feuerstein *et al.* (1985) have reported that endogenous NA can activate α_2-heteroreceptors on the 5-HT-containing terminals in the rabbit hippocampal slice when its synaptic concentration is increased by the uptake inhibitor, desipramine. It is noteworthy, however, that this effect does not appear to occur in brain slices prepared from the rat hypothalamus (Galzin *et al.*, 1984), rat hippocampus (Benikrane *et al.*, 1985), or rabbit cerebral cortex (Limberger *et al.*, 1986).

Attempts to confirm the physiological role of α_2-heteroreceptors *in vivo* have proven equally contentious. Using *in vivo* microdialysis, Tao and Hjorth (1992) demonstrated that although both systemic and local administration of idazoxan blocks the clonidine-induced reduction of 5-HT release from the ventral hippocampus of the rat, it has no effect on 5-HT release when given alone. This suggests that α_2-heteroreceptors on 5-HT–containing neurones are not tonically activated by endogenous NA. In contrast, Mongeau *et al.* (1993) have shown that increasing the synaptic concentration of NA (by administration of desipramine) reduces the duration of 5-HT-induced suppression of hippocampal CA3 pyramidal cell activity in the rat. However, it is interesting to note that the same group reported that idazoxan and yohimbine failed to alter the 5-HT–induced reduction in pyramidal cell activity. Obviously, further work will be required to establish the physiological relevance of the inhibitory α_2-adrenoceptors on central 5-HT nerve terminals.

α_2-Heteroreceptors also appear to exist on cholinergic nerve terminals in the CNS. Thus, NA has been shown to decrease, in a concentration-dependent manner, the evoked release of acetylcholine

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(ACh) from rat, guinea pig, and human brain slices (Paton and Vizi, 1969; Beani *et al.*, 1969; Vizi, 1979; see Beani and Bianchi, 1991, for a review). In addition, pharmacological manipulations that increase central NA levels are found to reduce ACh levels in the conscious guinea pig (Beani and Bianchi, 1970, 1991). Evidence that this subpopulation of α_2-heteroreceptors is activated tonically by endogenous NA is derived from two sources: first, locus coeruleus stimulation depresses ACh release in the ipsilateral cerebral cortex of the conscious guinea pig (Bianchi *et al.*, 1979) and, second, evoked ACh release is enhanced in brain slices obtained from rats in which the locus coeruleus has been lesioned (Vizi, 1980). However, more recently, Milusheva *et al.* (1994) have reported that cholinergic neurones in the rat hippocampus do not appear to possess α_2-heteroreceptors since α_2-adrenoceptor ligands had little effect on the evoked release of ACh from slices prepared from this brain region. Further studies will clarify these discrepancies.

2.2.2.2. *Control of blood pressure.* It is well documented that clonidine acts within the CNS to produce hypotension (Kobinger and Walland, 1967; Schmitt *et al.*, 1968; Katic *et al.*, 1972; Dollery and Reid, 1973). Evidence that this effect is attributable to stimulation of α_2-adrenoceptors comes largely from early pharmacological studies that demonstrated that other α_2-adrenoceptor agonists *mimic*, while α_2-adrenoceptor antagonists *attenuate*, the clonidine-induced reduction in blood pressure (see Berthelsen and Pettinger, 1977, and Timmermans and Van Zwieten, 1982, for reviews).

More recently, it has been suggested that the central hypotensive effect of clonidine, in fact, is mediated by nonadrenoceptor I_1 sites in the nucleus reticularis lateralis of the rostral ventrolateral medulla. Thus, other imidazoline-like compounds with low affinity for α_2-adrenoceptors (*e.g.*, cirazoline and imidazole-4-acetic acid) dose-dependently decreased blood pressure when injected directly into the nucleus reticularis lateralis of cats (Bousquet *et al.*, 1984) and rats (Ernsberger *et al.*, 1990). On the other hand, α-methylnoradrenaline, which is slightly more potent than clonidine as an α_2-adrenoceptor agonist (Starke *et al.*, 1975), had no effect following administration into the same region (Bousquet *et al.*, 1984). Similarly, the ability of a variety of imidazoline-like compounds to attenuate clonidine-induced hypotension was independent of their ability to block α_2-adrenoceptors (Karpannen, 1981; Ernsberger *et al.*, 1990; Tibiriça *et al.*, 1991). In addition, there is a good correlation between the ability of clonidine and related compounds to lower blood pressure when injected into the nucleus reticularis lateralis and their ability to displace the nonadrenoceptor component of $[^3H]$*p*-aminoclonidine binding in the bovine ventrolateral medulla (Ernsberger *et al.*, 1990).

Evidence that I_1 sites in the nucleus reticularis lateralis mediate the cardiovascular effects of clonidine, therefore, is very convincing. However, it does not entirely preclude the involvement of α_2-adrenoceptors in other brain regions. Indeed, Hieble and Kolpak (1993) have demonstrated that the *intravenous* administration of clonidine and guanabenz results in a reduction in blood pressure in the spontaneously hypertensive rat, an effect that was blocked by both idazoxan and SKF 86466. This is relevant because neither guanabenz nor SKF 86466 bind to I_1 sites. The precise anatomical location of the α_2-adrenoceptor-mediated reduction in blood pressure is unclear. Several groups have presented evidence implicating the nucleus tractus solitarius (De Jong and Nijkamp, 1976; Zandberg and De Jong, 1977; Kubo and Misu, 1981), a region that contains a high density of α_2-adrenoceptors in rat brain (Hudson *et al.*, 1992b). Other medullary structures (the dorsal motor nucleus of the vagus and the nucleus commissuralis), as well as interomediolateral cell column of the spinal cord, may also be involved (see Unnerstall *et al.*, 1984). In conclusion, it appears that both α_2-adrenoceptors and I_1 sites are involved in the central regulation of blood pressure.

2.2.2.3. *Motor activity.* The use of clonidine as an antihypertensive agent is associated with a number of side effects, one of which is sedation (Davidov *et al.*, 1967). This effect is also evident in animals, and clonidine has been shown to cause sedation in mice, rats, guinea pigs, gerbils, and cats (Laverty and Taylor, 1969; Laverty, 1970; Delbarre and Schmitt, 1971; Holman *et al.*, 1971; Kostowski and Malatynska, 1983). That this effect is mediated by α_2-adrenoceptors is well established since it is mimicked by other α_2-adrenoceptor agonists (Drew *et al.*, 1979) and attenuated by selective α_2-adrenoceptor antagonists (Clineschmidt *et al.*, 1979; Delini-Stula *et al.*, 1979; Drew *et al.*, 1979; Nomura *et al.*, 1980; Heal *et al.*, 1981), but not α_1- (Clineschmidt *et al.*, 1979; Drew *et al.*, 1979; Heal *et al.*, 1981) or β- (Drew *et al.*, 1979; Heal and Philpot, 1987) adrenoceptor antagonists. Furthermore, sedation can be evoked by intracerebroventricular injection of clonidine, and the sedation associated with its peripheral injection can by blocked by the intracerebroventricular injection of selective α_2-adrenoceptor antagonists; this suggests that the α_2-adrenoceptors that mediate clonidine-induced sedation are centrally located (Drew *et al.*, 1979).

The mechanism underlying the effect of α_2-adrenoceptor agonists on motor activity is thought to involve a reduction in noradrenergic transmission that is secondary to the activation of α_2-autoreceptors (Strömbom and Svensson, 1980; De Sarro *et al.*, 1987; Heal *et al.*, 1988a, 1989; Correa-Sales *et al.*, 1992; however, see Spyraki and Fibiger, 1982; Nassif *et al.*, 1983). More specifically, the stimulation of α_2-autoreceptors on the cell bodies of the locus coeruleus appears to play a key role in the induction of the sedative response (Weiss *et al.*, 1986; De Sarro *et al.*, 1987; Correa-Sales *et al.*, 1992; however, see Heal, 1990).

2.2.2.4. *Nociception.* The analgesic capacity of systemic, intrathecal, and epidural clonidine is well documented, both in animals (Paalzow, 1974; Yaksh and Reddy, 1981; Eisenach *et al.*, 1987; Ossipov *et al.*, 1989) and humans (Tamsen and Gordh, 1984; Coombs *et al.*, 1985). Furthermore, clonidine has been shown to potentiate morphine-induced analgesia (Fielding *et al.*, 1978; Tamsen and Gordh, 1984; Coombs *et al.*, 1985). Since antinociception is a property shared by a number of other α_2-adrenoceptor agonists (*e.g.*, medetomidine: Pertovaara *et al.*, 1990; dexmedetomidine: Fisher *et al.*, 1991; ST91: Yaksh and Reddy, 1981; Howe *et al.*, 1983; Tamsen and Gordh, 1984; Coombs *et al.*, 1985; metaraminol: Fleetwood-Walker *et al.*, 1985; guanfacine: Xu *et al.*, 1993; Puke *et al.*, 1994), it is very likely that this effect is mediated by α_2-adrenoceptors. Furthermore, the analgesic effects of clonidine and other α_2-adrenoceptor agonists are reduced or attenuated by yohimbine, rauwolscine, and idazoxan (Hynes *et al.*, 1983; Fleetwood-Walker *et al.*, 1985; Sullivan *et al.*, 1987; Fisher *et al.*, 1991; Kalso *et al.*, 1991; Lui *et al.*, 1993).

It should be noted, however, that stimulation of α_1-adrenoceptors also evokes antinociception (Reddy *et al.*, 1980; Howe *et al.*, 1983), and it has been suggested that clonidine-induced analgesia results from an interaction with both α_1- and α_2-adrenoceptor populations (see Paalzow and Paalzow, 1982; Hayes *et al.*, 1986). It is also notable that the type of test used to assess the antinociceptive effects of α_2-adrenoceptor ligands appears to influence the results obtained. Thus, Pertovaara *et al.* (1990) have demonstrated that while medetomidine produces dose-dependent analgesia in the formalin test in rats, it does not alter the tail flick latency or the tail biting latency in response to mechanical pain.

With regards to anatomical localisation, there is good evidence to suggest that the antinociceptive action of α_2-adrenoceptor agonists is mediated by receptors in the dorsal horn of the spinal cord (North and Yoshimura, 1984; Fleetwood-Walker *et al.*, 1985; Wikberg and Hajó, 1987; Sullivan *et al.*, 1987; Danzebrink and Gebhart, 1990). This is consistent with the presence of [^{3}H]RX821002 binding sites in this brain region (Hudson *et al.*, 1992b).

2.2.2.5. *Anxiety.* The role of α_2-adrenoceptors in anxiety-related behaviours was first suggested by the observation that administration of yohimbine or piperoxan to humans evoked intense anxiety and panic attacks (Goldenberg *et al.*, 1947; Soffer, 1954; Holmberg and Gershon, 1961; Margolis *et al.*, 1971; Charney *et al.*, 1983). This was substantiated by the proposal that clonidine itself is clinically useful in the treatment of certain types of anxiety (Hoehn-Saric *et al.*, 1981) and the demonstration that it abolishes the anxiogenic effects of yohimbine in humans (Charney *et al.*, 1983).

Outside the clinic, the situation is a little more complicated. The effect of α_2-adrenoceptor ligands on behaviour in animal models of anxiety has provided results as varied in number as the models themselves. For simplicity, these are summarised in Table 4. Such overt lack of consistency is difficult to account for and has cast doubts over the role of α_2-adrenoceptors in anxiety-related behaviours.

2.2.2.6. *Cognitive function.* There are several reports in the literature concerning the effects of α_2-adrenoceptor ligands on learning and memory processes. Clonidine improves spatial working memory in aged (Arnsten and Goldman-Rakic, 1984, 1985; Arnsten *et al.*, 1988) and young catecholamine-depleted monkeys (Brozoski *et al.*, 1979; Cia *et al.*, 1993). Guanfacine also improves cognitive function in aged monkeys (Arnsten *et al.*, 1988), confirming that this effect is mediated by α_2-adrenoceptors. In addition, clonidine has been noted to improve the memory of mice depleted of NA using a dopamine-β-hydroxylase inhibitor (Freedman *et al.*, 1979).

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Table 4. *Effects of α_2-Adrenoceptor Ligands on Anxiety-Related Behaviours in Various Animal Models*

α_2-Adrenoceptor ligand	Test	Species	Effect	Reference
Clonidine	elevated plus-maze	rat	anxiolytic	Handley and Mithani, 1984; Söderpalm and Engel, 1988
	potentiated startle	rat	anxiolytic	Davis *et al.*, 1977; Davis and Astrachan, 1981
	Geller Seifter conflict	rat	anxiolytic	Kruse *et al.*, 1981
	Geller Seifter conflict	mouse	no effect	Venault *et al.*, 1993
	Vogel conflict	rat	anxiolytic	La Marca and Dunn, 1994
Medetomidine	elevated plus-maze	rat	no effect	Rago *et al.*, 1990
	Vogel conflict	rat	no effect	La Marca and Dunn, 1994
Guanfacine	Vogel conflict	rat	no effect	La Marca and Dunn, 1994
Guanabenz	Vogel conflict	rat	no effect	La Marca and Dunn, 1994
Yohimbine	elevated plus-maze	rat	anxiogenic	Handley and Mithani, 1984; Pellow *et al.*, 1985a; File, 1987; Redfern and Williams, 1990
	potentiated startle	rat	anxiogenic	Davis *et al.*, 1977
	Geller Seifter conflict	mouse	anxiogenic	Venault *et al.*, 1993
	Vogel conflict	rat	anxiolytic	Gower and Tricklebank, 1988
	Vogel conflict	rat	no effect	La Marca and Dunn, 1994
	social interaction	rat	anxiogenic	Pellow *et al.*, 1985b
	shock-induced ultrasonic vocalisation	rat	anxiolytic	De Vrey *et al.*, 1993
Rauwolscine	Vogel conflict	rat	anxiolytic	La Marca and Dunn, 1994
Piperoxan	elevated plus-maze	rat	anxiogenic	Handley and Mithani, 1984
	potentiated startle	rat	anxiogenic	Davis *et al.*, 1977
	Vogel conflict	rat	no effect	La Marca and Dunn, 1994
Idazoxan	elevated plus-maze	rat	anxiogenic	Handley and Mithani, 1984
	elevated plus-maze	rat	no effect	Söderpalm and Engel, 1988; File, 1987; Redfern and Williams, 1990; Moser, 1989
	elevated plus-maze	mouse	no effect	Durcan *et al.*, 1989
	Geller Seifter conflict	mouse	anxiogenic	Venault *et al.*, 1993
	Vogel conflict	rat	no effect	Heilig *et al.*, 1989
	Vogel conflict	rat	anxiolytic	La Marca and Dunn, 1994; De Vrey *et al.*, 1993
	social interaction	rat	no effect	File, 1987
	shock-induced ultrasonic vocalisation	rat	anxiolytic	De Vrey *et al.*, 1993
Atipamezole	elevated plus-maze	rat	no effect	Kauppila *et al.*, 1991
	elevated plus-maze	mouse	no effect	Durcan *et al.*, 1989

α_2-Adrenoceptor antagonists also have a positive effect on cognitive function. Thus, yohimbine and idazoxan facilitate memory retrieval processes in the rat (Sara, 1985; Sara and Devauges, 1989). In addition, chronic treatment of aging rats with piperoxan retards the decline in memory function (Zornetzer, 1985).

The ability of α_2-adrenoceptor agonists *and* antagonists to enhance cognitive function may appear somewhat puzzling, given their opposing actions on α_2-adrenoceptor function. The solution to this apparent paradox lies with the localisation of the receptor population affected by these ligands. Thus, clonidine-induced improvements in spatial memory are thought to be mediated by postsynaptic α_2-adrenoceptors in the prefrontal cortex (Cia *et al.*, 1993). In contrast, the effect of the α_2-adrenoceptor antagonists appears to be due to the increase in NA release, which is secondary to the blockade of α_2-autoreceptors. Thus, locus coeruleus stimulation mimics the effects of idazoxan, yohimbine, and piperoxan on memory retrieval (Zornetzer, 1985; Sara and Devauges, 1988).

2.2.2.7. *Other functions.* Evidence has also been presented implicating central α_2-adrenoceptors in the control of body temperature (Laverty and Taylor, 1969; Von Voigtlander *et al.*, 1978; Lin *et al.*, 1981; Maskrey *et al.*, 1970), pupil diameter (Berridge *et al.*, 1983; Heal *et al.*, 1988b), endocrine secretion (Plotsky *et al.*, 1989), sexual behaviour (Vincent *et al.*, 1989), appetite (Goldman *et al.*, 1985), and glucoregulation (Smythe and Edwards, 1991).

2.2.2.8. *Function of α_2-adrenoceptor subtypes.* As discussed in the preceding sections, central α_2-adrenoceptors appear to mediate a number of specific functions in the brain. Assigning these functions to a particular α_2-adrenoceptor subtype is difficult, however, given the present lack of subtype-selective ligands. Nevertheless, Trendelenburg and co-workers recently have provided convincing pharmacological evidence to suggest that the α_2-auto- and heteroreceptors mediating the release of NA and 5-HT, respectively, belong to the $H\alpha_{2A}$ subclass in rabbits and the $R\alpha_{2A}$ subclass in rats (Trendelenburg *et al.*, 1993, 1994). Similarly, the α_2-autoreceptor in the human cerebral cortex has the pharmacological characteristics of the $H\alpha_{2A}$-adrenoceptor subtype (Raiteri *et al.*, 1992). In addition, the α_2-autoreceptors on the cell bodies of the rat locus coeruleus appear to belong to the $R\alpha_{2A}$ subclass (Wamsley *et al.*, 1992; Zeng and Lynch, 1991; McCune *et al.*, 1993; Nicholas *et al.*, 1993; Scheinin *et al.*, 1994).

The sedative and cognitive actions of α_2-adrenoceptor agonists are mediated, at least in part, via an interaction with the somatodendritic autoreceptors (see Sections 2.2.2.3 and 2.2.2.6), suggesting $R\alpha_{2A}$-adrenoceptors are involved in the control of motor behaviour and learning and memory processes. In partial confirmation of this, Millan *et al.* (1994) have demonstrated that in rats and mice, the α_2-adrenoceptor mediating the sedative effect of α_2-adrenoceptor agonists has the pharmacological characteristics of the $R\alpha_{2A}$ subtype.

Some progress has also been made in determining the α_2-adrenoceptor subtype(s) mediating antinociception. Xu *et al.* (1993) have demonstrated that in rats, the analgesic action of clonidine (which does not discriminate between $R\alpha_{2A}$, α_{2B}, and α_{2C} subtypes) is greater than that of the $R\alpha_{2A}$-selective agonist, guanfacine. Since the spinal cord in this species contains $R\alpha_{2A}$ (96%) and α_{2C} (4%) receptors, but no α_{2B} receptors (Uhlén *et al.*, 1992), this group has suggested that both $R\alpha_{2A}$- and α_{2C}-adrenoceptors are involved in the antinociceptive effect. Recently, this conclusion has been confirmed by a more extensive study performed by Millan *et al.* (1994).

3. THE DISCOVERY OF I_2 SITES

Coupry *et al.* (1987) was the first group to demonstrate that idazoxan binds with high (nanomolar) affinity to a distinct population of nonadrenoceptor binding sites. Thus, in basolateral membranes from rabbit kidney, [^{3}H]idazoxan labelled 3.5 times more sites than the nonimidazoline α_2-adrenoceptor antagonist, [^{3}H]rauwolscine. Furthermore, only 25% of [^{3}H]idazoxan binding was displaceable by adrenaline, while α-adrenergic compounds containing an imidazoline ring (*e.g.*, cirazoline) completely inhibited the binding of [^{3}H]idazoxan.

Since 1987, idazoxan has been shown to bind to nonadrenoceptor sites in a variety of peripheral tissues from a number of different species (Langin and Lafontan, 1989; Michel, M. C. *et al.*, 1989; Vigne *et al.*, 1989; Zonnenschein *et al.*, 1990; Yablonski and Dausse, 1991). In addition, these sites have been identified in brain tissue derived from the rat (Wikberg, 1989; Brown *et al.*, 1990), guinea pig (Wikberg, 1989), rabbit (Convents *et al.*, 1989; Hamilton *et al.*, 1991), and human (Convents *et al.*, 1989; De Vos *et al.*, 1991). In accordance with the original work of Coupry *et al.* (1987), these studies consistently demonstrated that:

1. [^{3}H]idazoxan labels more sites than other nonimidazoline α_2-adrenoceptor antagonists,
2. a component of the specific [^{3}H]idazoxan binding was insensitive to displacement by catecholamines, and
3. this residual component was sensitive to compounds containing an imidazoline(-like) moiety.

Since very similar results had been obtained previously for the α_2-adrenoceptor agonist, clonidine (*i.e.*, it was shown to bind with nanomolar affinity to a population of nonadrenoceptor sites that were sensitive to imidazoline(-like) compounds; see Ernsberger *et al.*, 1987, 1990; Bricca *et al.*, 1989), it was initially assumed that both clonidine and idazoxan labelled the same population of nonadrenoceptor sites. It is now clear that this is *not* the case since there are distinct differences in the pharmacological characteristics of the two sites (see Michel and Insel, 1989, for a review). Most notable is the relatively low (micromolar) affinity of clonidine for the idazoxan-labelled site

(Michel, M. C. *et al.*, 1989; Zonnenschein *et al.*, 1990; Wikberg, 1989). Idazoxan itself is less able to discriminate between the two populations of nonadrenoceptor sites. Thus, it has an equally high affinity for both sites in human brain (see Bricca *et al.*, 1989; Convents *et al.*, 1989), while in rat brain, its affinity for the clonidine-labelled site is 20-fold lower than its affinity for its own site (see Ernsberger *et al.*, 1990; Hudson *et al.*, 1992a).

Another distinguishing feature of the two sites relates to their affinity for certain guanidine-like compounds, which bind to the idazoxan-labelled sites at low (nanomolar) concentrations (Michel, M. C. *et al.*, 1989; Vigne *et al.*, 1989; Zonnenschein *et al.*, 1990), but do not recognise the sites labelled by clonidine (Ernsberger *et al.*, 1990; Wikberg *et al.*, 1992; however, see De Vos *et al.*, 1994). Conversely, compounds such as cimetidine and imidazole-4-acetic acid have an appreciably higher affinity for the clonidine-sensitive site (see Ernsberger *et al.*, 1987; Wikberg and Uhlén, 1990).

On the basis of these differences, it was suggested at the First International Symposium on Imidazoline-Preferring Receptors in 1992 that the clonidine-labelled sites should be classified as I_1 and idazoxan-labelled sites as I_2 (see Michel and Ernsberger, 1992). The "I" prefix was chosen in view of the sensitivity of both sites to compounds containing an imidazoline or an imidazoline-like moiety. However, it is now recognised that a number of nonimidazoline compounds (*e.g.*, guanidine and its analogues) can also bind with high affinity to I sites (Vigne *et al.*, 1989; Zonnenschein *et al.*, 1990) and, in contrast, certain compounds with an imidazoline(-like) structure (*e.g.*, the α_2-adrenoceptor antagonist, RX821002; see Table 1) have a very low affinity for these sites (Mallard *et al.*, 1992). Prior to the symposium, there was no general consensus concerning the terminology applied to these nonadrenoceptor sites and, inevitably, individual groups used their own acronyms. This obviously led to some confusion in the literature, particularly in the case of I_2 sites, which were referred to using at least six different terms, including nonadrenoceptor idazoxan binding sites, imidazoline-guanidinium receptive sites, and imidazoline-preferring receptors. The "I" nomenclature is now (almost) universally recognised, however.

The distinction between the I_1 and I_2 sites has been further emphasised by the recent demonstration that the distribution of each site in the human brain is distinct (De Vos *et al.*, 1994).

3.1. Subclassification of I_2 Sites

Evidence that peripheral and central I_2 sites are heterogeneous in nature originally was derived from radioligand-binding studies using amiloride. This compound displaces [^{3}H]idazoxan binding from I_2 sites in rabbit kidney (Coupry *et al.*, 1989), adipocytes (Langin and Lafontan, 1989), urethra (Yablonski and Dausse, 1991), and liver (Tesson *et al.*, 1990) with high affinity (30–160 nM), while concentrations 10–100 times in excess of this are required to displace I_2 site binding in the kidney (Michel and Insel, 1989; Michel, M. C. *et al.*, 1989; Vigne *et al.*, 1989), liver (Tesson *et al.*, 1990; Zonnenschein *et al.*, 1990), and brain (Wikberg and Uhlén, 1990; Miralles *et al.*, 1993a) of nonrabbit species.

These pharmacological differences initially were attributed simply to species differences rather than genuine heterogeneity, a view fuelled by the observation that rabbit I_2 sites have a somewhat higher affinity for clonidine than nonrabbit I_2 sites (Yablonsky *et al.*, 1988; Langin and Lafontan, 1989). However, evidence has now been advanced that indicates that I_2 sites with a high and low affinity for amiloride are coexpressed not only within the same species (Diamant *et al.*, 1992), but also within the same tissue (Renouard *et al.*, 1993). Furthermore, Lanier *et al.* (1993) have isolated two peptides from rat liver mitochondrial membranes with the ligand recognition properties of the I_2 sites; only one of these is sensitive to amiloride, however. Those I_2 sites with a high and low affinity for amiloride tentatively have been termed I_{2A} and I_{2B}, respectively (see Parini, 1995).

Radioligand-binding studies using the imidazoline-containing compound, naphazoline, also indicate that I_2 sites are heterogeneous in nature. Thus, in rat, rabbit, and dog liver (Stewart *et al.*, 1992), as well as rat kidney (MacKinnon *et al.*, 1993) and rabbit cerebral cortex (Renouard *et al.*, 1993), this compound displaces I_2 binding from two distinct sites. In addition, the existence of two I_2 site subtypes is indicated by the observation that (+)-medetomidine has a 12-fold higher affinity for I_2 sites in the guinea pig cerebral cortex than (−)-medetomidine, while the I_2 sites of the guinea pig ileum have an equal affinity for both stereoisomers (Wikberg *et al.*, 1991).

It presently is unclear how the subtypes identified with naphazoline and medetomidine relate to those identified with amiloride, however. According to the work presented by Renouard *et al.* (1993), the high- and low-affinity naphazoline sites in rabbit cerebral cortex have a correspondingly high and low affinity for amiloride. In contrast, the results obtained by MacKinnon *et al.* (1993) indicate that *both* sites identified with naphazoline in rat kidney have a low affinity for amiloride. Similarly, the two I_2 sites in guinea pig cortex distinguished by the medetomidine stereoisomers appear to correspond to the low-affinity amiloride site (Wikberg *et al.*, 1991).

Wikberg's group has proposed that I_2 sites exist in two or more interacting or interconvertible forms. The evidence for this was generated from two studies. In the first, displacement of [^{3}H]idazoxan binding to I_2 sites in guinea pig kidney membranes was biphasic for 22 of 29 competing ligands (Wikberg *et al.*, 1992). Since the proportion of high- and low-affinity sites defined by these compounds varied widely (from 50:50 to 8:92), it was suggested that the two sites could not be independent, noninteracting sites, but instead were dynamically formed or modulated in the presence of the drug. In their second study, kinetic analysis of [^{3}H]idazoxan binding to I_2 sites in guinea pig liver and kidney revealed the presence of three sites with distinct association and dissociation constants (Wikberg, 1995). The relative proportion of each site was different in the association and dissociation experiments, however, a result that appears to corroborate with the conclusions arrived at in their first study (Wikberg *et al.*, 1992).

3.2. I_2-Selective Ligands

Although idazoxan has been immensely valuable in the study of I_2 sites, its use is compromised by the fact that it binds with equally high affinity to α_2-adrenoceptors (Mallard *et al.*, 1992). For this reason, a great deal of research effort has been invested in developing compounds with improved I_2:α_2 selectivity, and the fruits of this labour are now in evidence.

One approach that is commonly used to develop novel ligands for the study of specific receptor systems is the chemical modification of the structure of those ligands already available. Modification of the structure of idazoxan {2-[2-(1,4-benzodioxanyl)]-2-imidazoline} has been particularly productive since it has provided three compounds, RX801023 [6-fluoro-2-(1,4-benzodioxan-2-yl)-2-imidazoline], RX821029 [2-(1,3-benzodioxanyl)-2-imidazoline], and 2-BFI [RX801077; 2-(2-benzofuranyl)-2-imidazoline], with an improved I_2:α_2 selectivity profile. Thus, in brain tissue derived from a variety of mammalian species, each of these compounds has a high (nanomolar) affinity for I_2 sites, while their affinity for α_2-adrenoceptors is 29–2874 times lower (Hudson *et al.*, 1992a; Mallard *et al.*, 1992; Hudson *et al.*, 1994, 1995).

Menargues *et al.* (1994) have also described a novel I_2-selective ligand, LSL 60101 (2-(2-benzofuranyl)-imidazole), which is very similar in structure to 2-BFI. In radioligand-binding studies, this compound was found to be ~300 times more selective for I_2 sites than α_2-adrenoceptors, although its affinity for these sites is slightly lower than that of idazoxan and 2-BFI. More recently, BU224 [2-(4,5-dihydroimidaz-2-yl)-quinoline] and BU239 [2-(4,5-dihydroimidaz-2-yl)-quinoxaline] have been identified as selective ligands for I_2 sites in rabbit brain (Hudson *et al.*, 1994). In this tissue, both compounds are ~4000 times more selective for I_2 sites than α_2-adrenoceptors. Furthermore, their affinity for I_2 sites is in the low nanomolar range.

The chemical structures of idazoxan and the I_2-selective ligands described above are presented in Fig. 1 and details of their selectivity profile are summarised in Table 5.

With the exception of 2-BFI, no information is available yet regarding the possible interaction of the I_2-selective compounds with other neurotransmitter systems. Extensive radioligand-binding studies performed shortly after its development have shown that at concentrations up to 10^{-5} M, 2-BFI does not bind to any of the classical neurotransmitter receptors. In addition, it had no appreciable affinity for various channel proteins and peptide binding sites (A. Hudson, personal communication). If the same is true of BU224 and BU239, then there is little doubt that all three compounds will be very useful for examining the pharmacology and function of central I_2 sites.

3.3. Localisation of I_2 Sites in the Brain

I_2 sites have been identified in the brain tissue of every mammalian species thus far examined (Wikberg, 1989; Convents *et al.*, 1989; Brown *et al.*, 1990; De Vos *et al.*, 1991; Hamilton *et al.*,

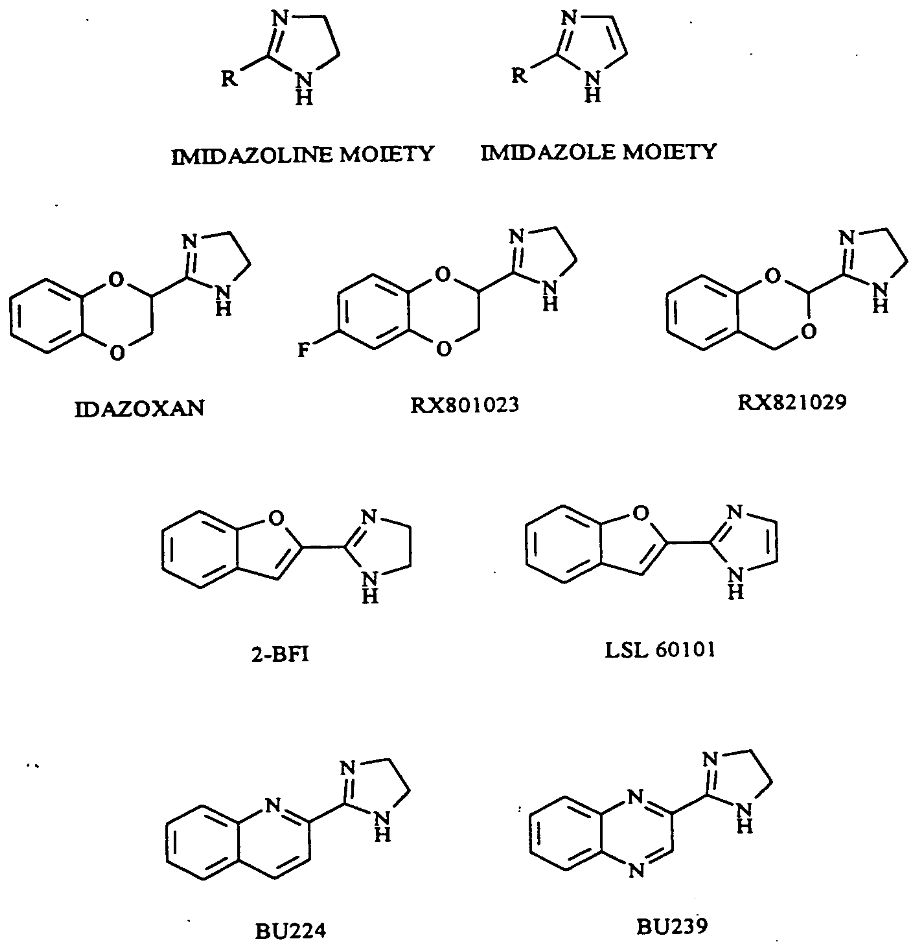


Fig. 1. Chemical structures of idazoxan and various imidazole- or imidazoline-containing I_2-selective ligands.

1991). To examine the localisation of these sites in detail requires the availability of an I_2-selective radioligand. As discussed in the preceding section, there are a number of ligands that show good selectivity for I_2 sites over α_2-adrenoceptors. One of these, 2-BFI, recently has been tritiated, and Lione *et al.* (1995) have shown that it retains its high affinity and selectivity for I_2 sites in rabbit brain. These findings suggest that [^{3}H]2-BFI will be useful for the determination of the autoradiographic localisation of I_2 sites. In the meantime, information regarding the distribution of central I_2 sites must be derived indirectly from work carried out using [^{3}H]idazoxan.

Mallard *et al.* (1992) have determined the localisation of I_2 sites in rat brain using [^{3}H]idazoxan in the presence of micromolar concentrations of various α_2-adrenoceptor ligands to preclude binding to α_2-adrenoceptors. The results obtained indicate that I_2 sites are present, in varying concentrations, in each of the 22 brain regions examined. High densities were observed in the area postrema, arcuate nucleus, interpeduncular nucleus, and ependyma. In the remaining regions, moderate levels of I_2 sites were present in the hippocampal fissure and superior colliculus, while the cortex, caudate nucleus, lateral and ventromedial hypothalamic nuclei, ventromedial thalamus, central gray, inferior colliculus, lateral habenula, and nucleus of the solitary tract contained low (but detectable) levels of I_2 sites.

In the human brain, [^{3}H]idazoxan, in the presence of 5 μM adrenaline, was used to determine the autoradiographic distribution of I_2 sites (De Vos *et al.*, 1991). The highest density of sites occurred in the basal ganglia (caudate nucleus, putamen, and globus pallidus) and substantia nigra. Moderate levels were noted in the cerebral cortex, hippocampus, amygdala, inferior olivary nucleus,

Table 5. *Affinity of Idazoxan and Various I_2-Selective Ligands for α_2-Adrenoceptors and I_2 Sites in Brain Tissue*

Compound	Species	Affinity[1] (nM) α_2	I_2	Selectivity ratio $I_2{:}\alpha_2$	Reference
Idazoxan	rat	11.2	8.5	1.3	Hudson *et al.*, 1992a
RX801023	rat	260.7	4.8	54	Mallard *et al.*, 1992
	rabbit	142.8	4.9	29	Hudson *et al.*, 1994
RX820129	rat	244.1	1.8	136	Hudson *et al.*, 1992a
	rabbit	436.2	2.7	161.6	Hudson *et al.*, 1992a
	guinea pig	171.5	3.7	46	Hudson *et al.*, 1995
	human	>1000	8.9	>112.4	Hudson *et al.*, 1992a
2-BFI	rat	1860.0	1.2	1550	Hudson *et al.*, 1995
	rabbit	3736.0	1.3	2874	Hudson *et al.*, 1995
	guinea pig	1600	1.9	842	Hudson *et al.*, 1995
LSL 60101	—[2]	350	14	25	Menargues *et al.*, 1994
BU224	rabbit	12100	2.9	4172	Hudson *et al.*, 1994
BU239	rabbit	7296	1.9	3840	Hudson *et al.*, 1994

[1] With the exception of LSL 60101, the affinity of each ligand for α_2-adrenoceptors and I_2 sites was determined according to their ability to displace [^{3}H]RX821002 from α_2-adrenoceptors and [^{3}H]idazoxan from I_2 sites.

[2] Menargues *et al.* (1994) do not state the species used in the radioligand binding studies referred to in this abstract.

and ependyma, while the mediodorsal and ventrolateral thalamic nuclei, as well as the cerebellum, contained low levels. These results indicate that there is some overlap in the distribution of I_2 sites between human and rat brain. It is interesting to note, however, that the regions with the highest I_2 site density in human and rat brain are different (De Vos *et al.*, 1991; Mallard *et al.*, 1992).

3.4. Functional Role of Central I_2 Sites

3.4.1. *Endogenous Ligand*

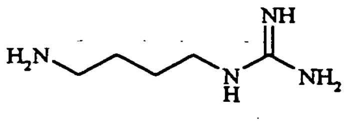
As detailed in the preceding section, the autoradiographic distribution of I_2 sites in the rat brain is such that these sites are localised in several distinct regions, including the arcuate nucleus and the interpeduncular nucleus (Mallard *et al.*, 1992). Since these regions contain high levels of peptides and hormones, it has been proposed that the endogenous ligand for I_2 sites may be peptidergic or hormonal in nature. However, MacKinnon *et al.* (1992b) have screened some 30 naturally occurring peptides and hormones and found that none had a high affinity for I_2 sites in the rabbit renal cortex.

Alternative theories regarding the endogenous ligand for I_2 sites have focussed on a "clonidine-displacing substance" (CDS), which is a bioactive substance purified from bovine brain (Atlas and Burstein, 1984) and postulated to be the endogenous ligand for I_1 sites (Ernsberger *et al.*, 1986; Meeley *et al.*, 1986). The demonstration that CDS also competes for nonadrenoceptor [^{3}H]idazoxan binding with a relatively high potency in a variety of tissues led to the proposal that it also serves as the native ligand for I_2 sites (Coupry *et al.*, 1989; Parini *et al.*, 1989; Zonnenschein *et al.*, 1990; Singh *et al.*, 1992).

Recently, Li *et al.* (1994) have extracted and purified a CDS from bovine brain, and using mass spectroscopy, have identified it as *agmatine* (Fig. 2). Since agmatine shares some of the functional properties of CDS and since it also displaces I_1, I_2, and α_2-adrenoceptor binding from various bovine tissues in a manner analogous to CDS, Li *et al.* (1994) have suggested that agmatine is the universal endogenous ligand for I sites. This proposal has been the target of some criticism, however (Altas, 1994; see also Reis *et al.*, 1994).

3.4.2. *Feeding*

Idazoxan stimulates feeding in rats (Sleight *et al.*, 1988), an effect that originally was assumed to be attributable to its classical α_2-adrenoceptor blocking actions. However, Jackson *et al.* (1991) have shown that RX811059 and RX821002 (Stillings *et al.*, 1985; see Table 1), which are potent



AGMATINE

Fig. 2. Chemical structure of agmatine, the proposed endogenous ligand for I_1 and I_2 sites.

α_2-adrenoceptor antagonists with a very low affinity for I_2 sites (see Mallard *et al.*, 1992), do not alter food intake in rats, suggesting that idazoxan-induced hyperphagia is mediated by I_2 sites rather than α_2-adrenoceptors.

In agreement with this, the I_2-selective ligand, LSL 60101 (see Table 5) increased food intake in freely feeding rats (Menargues *et al.*, 1994). This effect persisted in rats pretreated with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, which preferentially alkylates central α_2-adrenoceptors (Miralles *et al.*, 1993b) and provides further evidence that LSL 60101-induced hyperphagia is selectively mediated by I_2 sites. However, it is notable that in this study, yohimbine and RX821002 were also shown to elicit an increase in food intake, which suggests that this effect is mediated, in part, by α_2-adrenoceptors (Menargues *et al.*, 1994).

Presently, it is unclear whether peripheral and/or central I_2 sites mediate feeding behaviour in the rat. Thus, idazoxan was given systemically in the study of Jackson *et al.* (1991), and it is well established that this compound can cross the blood–brain barrier (Lewis *et al.*, 1988). However, the high density of I_2 sites in the arcuate nucleus and area postrema (Mallard *et al.*, 1992) suggests that central I_2 sites are important, since these brain regions are known to be intimately involved in the control of gustatory processes (Beck *et al.*, 1990a,b; Sanacora *et al.*, 1990; Blundell, 1991).

3.4.3. *Interaction with Monoamine Oxidase*

Using tissue homogenates from rabbit and human liver, Tesson *et al.* (1991) was the first group to show that I_2 sites are localised to the mitochondrial fraction, a finding that subsequently was confirmed in a wider range of tissues, including brain, from rabbit, cow, and human (Tesson and Parini, 1991; Regunathan and Reis, 1992; Tesson *et al.*, 1992). The link between I_2 sites and monoamine oxidase (MAO) was based on an extension of this work. Thus, Tesson *et al.* (1991) demonstrated that [^{3}H]idazoxan binding to I_2 sites was positively correlated with MAO activity in the outer mitochondrial membrane, but negatively correlated with cytochrome oxidase activity in the inner mitochondrial membrane.

Further evidence of an interaction between I_2 sites and MAO has been presented by Olmos *et al.* (1993). This group demonstrated that chronic (but not acute) treatment with the MAO inhibitors, clorgyline and pargyline, down-regulated I_2 sites in rat brain. In addition, Sastre and García-Sevilla (1993) observed an increase in the density of I_2 sites in the aged human brain, which correlated well with the concurrent increase in the MAO-B binding. Since there was no change in the density of MAO-A binding sites with age, this suggests that the link between I_2 sites and MAO relates specifically to the MAO-B isoform. Indeed, Sastre and García-Sevilla (1993) have shown that there is a highly significant correlation between the density of I_2 and MAO-B binding sites in human brain in eight different brain regions, but no correlation between I_2 and MAO-A binding sites. Using autoradiographic binding data obtained by other groups, they also found a good correlation between the distribution of I_2 and MAO-B sites in rat brain (15 regions), but a poor correlation between I_2 and MAO-A sites (see also Renouard *et al.*, 1993).

The functional relevance of the I_2-MAO interaction is presently unclear. However, it is interesting to note that several I_2-selective compounds have been shown to alter monoamine release and turnover in the brain. Thus, RX821029 increases the extracellular concentration of dopamine in the rat striatum, an effect that is accompanied by a reduction in the major dopamine metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, as well as the 5-HT metabolite, 5-hydroxyindoleacetic acid (Lalies and Nutt, 1993). In addition, 2-BFI has been shown to increase NA release from the hippocampus and frontal cortex of the rat (Lalies and Nutt, 1995). Whether I_2 sites are physiologically important in the regulation of MAO activity remains to be determined.

Recently, Carpéné and Lafontan (1995) have examined the effect of a range of compounds on MAO activity in rat liver and adipocytes. Although various ligands with known affinity for I_2 sites (cirazoline, RX821029, and 2-BFI) *did* inhibit the enzyme, it is of interest that the potency of each of the three compounds did *not* correlate with their affinity for I_2 sites. However, this may relate to the fact that the assay used to examine MAO activity was the oxidation of tyramine, a substrate for both MAO-A and MAO-B (Youdim and Finberg, 1991); as discussed above, it appears that the interaction between I_2 sites and MAO specifically involves the MAO-B isoform (Renouard *et al.*, 1993; Sastre and García-Sevilla, 1993). It remains to be determined if I_2-selective compounds inhibit MAO-A and/or MAO-B activity in brain tissue and, more importantly, whether this relates to their affinity for central I_2 sites.

Finally, it is worth mentioning that it is unlikely that all I_2 sites are localised to mitochondrial membranes, at least in the periphery. Thus, several groups have reported that I_2 sites are also present in the plasmalemma (Coupry *et al.*, 1989; Zonnenschein *et al.*, 1990; Yablonski and Dausse, 1991). It is unclear whether this also applies to the CNS.

3.4.4. *Regulation of Glial Fibrillary Acidic Protein Expression*

Sastre and García-Sevilla (1993), who demonstrated age-dependent increases in the density of I_2 sites and MAO-B sites in human brain (see Section 3.4.3), have interpreted their results to indicate that central I_2 sites are localised, at least in part, to glial cells. Thus, in humans, the increase in MAO-B activity with age is thought to be entirely attributable to astrocytic hypertrophy (Nakamura *et al.*, 1990; Arai *et al.*, 1985). In addition, MAO-B in the monkey and human brain is located mainly in glial cells and 5-HT-containing neurones (Westlund *et al.*, 1985, 1988). In support of this, Regunathan *et al.* (1993) have reported that rat cortical astrocytes in culture express I_2 sites, while cortical neurones are devoid of these sites.

The precise function of glial I_2 sites in the brain is unclear. However, it appears to involve *glial fibrillary acidic protein* (GFAP), the main constituent of the intermediate filaments of astrocytes. Thus, idazoxan has been shown to dose-dependently increase the levels of GFAP mRNA in rat cortical astrocytes (Regunathan *et al.*, 1993), and Olmos *et al.* (1994) have demonstrated that chronic administration of compounds with high affinity for I_2 sites causes a parallel increase in the density of I_2 sites and GFAP. It is possible, therefore, that I_2 sites modulate the activity of this protein in central glial cells. This is an intriguing possibility, since idazoxan has been reported to have neuroprotective properties in models of focal (Maiese *et al.*, 1992) and global (Gustafson *et al.*, 1989) cerebral ischemia, and astrocytic proliferation in response to brain injury is thought to function as a protective mechanism (see Kindy *et al.*, 1992). It is also interesting to note that in patients suffering from Alzheimer's disease, which is also associated with astrocytic hypertrophy (Frederickson, 1992), elevated levels of I_2 sites and GFAP in the brain have been reported (Le Prince *et al.*, 1993; Ruiz *et al.*, 1993).

3.4.5. *Cerebral Glucose Use*

The quantitative autoradiographic [^{14}C]2-deoxyglucose procedure is used to measure the rate of local cerebral glucose use (LCGU; Sokoloff *et al.*, 1977; see McCulloch, 1982, for a review). Since the rate of glucose use in any brain region is directly proportional to its level of functional activity, this procedure can be used to predict those structures functionally important in drug action by examining the regional patterns of glucose use following its administration. For this reason, we recently have evaluated the effect of the I_2-selective ligand, 2-BFI, on LCGU in the rat using the [^{14}C]2-deoxyglucose procedure (French *et al.*, 1995b).

The results obtained indicate that acute administration of 2-BFI (1, 3, and 10 mg kg^{-1} i.v.) altered LCGU in only 10 of the 77 structures examined (Table 6). The changes observed in the arcuate nucleus and ventromedial thalamus are particularly interesting since both regions are thought to be involved in the regulation of food intake (Beck *et al.*, 1990a,b; Sanacora *et al.*, 1990; Blundell, 1991) and, as discussed in Section 3.4.2, compounds with high affinity for I_2 sites alter feeding behaviour in the rat (Jackson *et al.*, 1991; Menargues *et al.*, 1994). The significance of 2-BFI-induced alterations in LCGU in certain components of the septohippocampal formation (the molecular layer of the CA2 field of the hippocampus, the subiculum and the fornix) is unclear. However, given that

Table 6. *2-BFI-Induced Alterations in Local Cerebral Glucose Use in the Rat*

Structure	Saline	2-BFI (mg kg^{-1} i.v.) 1	3	10
Inferior colliculus	126 ± 8	126 ± 11	159 ± 6^1	131 ± 6
Superior olive	93 ± 4	99 ± 4	111 ± 6^1	90 ± 3
Hippocampal CA2 moleculare	82 ± 3	76 ± 3	77 ± 4	68 ± 1^1
Subiculum	76 ± 3	75 ± 3	77 ± 4	96 ± 3^1
Fornix	60 ± 2	61 ± 4	62 ± 2	80 ± 4^1
Somatosensory cortex	103 ± 3	96 ± 4	93 ± 2^1	89 ± 2^1
Motor cortex	101 ± 3	95 ± 4	94 ± 3	89 ± 1^1
Mediodorsal thalamus	72 ± 2	64 ± 2	69 ± 3	81 ± 3^1
Ventromedial thalamus	88 ± 2	92 ± 4	92 ± 3	103 ± 2^1
Arcuate nucleus	46 ± 2	59 ± 2^1	55 ± 1^1	–

Each value represents local cerebral glucose use (μmol/100 g/min) ± SEM (n = 5/group). Statistical analysis was performed using one-way analysis of variance followed by Newman–Keuls multiple range test: $^1P < 0.05$.

idazoxan has been shown to facilitate memory retrieval in the rat (Sara and Devauges, 1989) and since the septohippocampal formation is thought to play a pivotal role in learning and memory processes (Gray, 1981), it may indicate that I_2 sites in this region are involved in cognitive function. Obviously, further behavioural studies are required to confirm this.

We have also examined the effect of idazoxan (0.3–3 mg kg^{-1} s.c.; French *et al.*, 1992, 1995a) and RX811059 (0.3–3 mg kg^{-1} s.c.; French *et al.*, 1993) on LCGU in the rat. Interestingly, the pattern of changes evoked by idazoxan is most closely replicated by RX811059 and displays only limited similarity with that obtained for 2-BFI. Indeed, when the relationship between the effects of each of the three compounds was statistically assessed using the method of Ford *et al.* (1985), there was a significant correlation between the effect of idazoxan and RX811059 (r = 0.37; $P < 0.01$; Fig. 3A), but not idazoxan and 2-BFI (r = 0.16; Fig. 3B) or RX811059 and 2-BFI (r = −0.11; Fig. 3C). This suggests that changes in LCGU following administration of idazoxan primarily reflect the blockade of α_2-adrenoceptors as opposed to its interaction with I_2 sites.

3.5. The I_2 Site—Is It Distinct From the α_2-Adrenoceptor?

Michel, M. C. *et al.* (1989) have published evidence for the presence of I_2 sites in rat and human kidney, as well as human platelets and myometrium. Since each of these tissues also contains α_2-adrenoceptors, this group proposed that I_2 sites are closely affiliated with, or even part of, the α_2-adrenoceptor. This view was substantiated by the observation that three cell lines devoid of α_2-adrenoceptors were also lacking in I_2 sites (Michel, M. C. *et al.*, 1989).

The possibility of a direct association between α_2-adrenoceptors and I_2 sites has now been ruled out on the basis of an abundance of experimental evidence. First, tissues that express α_2-adrenoceptors, but not I_2 sites, have now been identified and *vice versa* (Michel *et al.*, 1990; Zonnenschein *et al.*, 1990). Similarly, Angel *et al.* (1995) have demonstrated that in Chinese hamster ovary cells transfected with the genes encoding the human $H\alpha_{2A}$, α_{2B}, and α_{2C} subtypes, I_2 sites are not coexpressed (see also Michel *et al.*, 1990). In addition, partially purified α_2-adrenoceptors and I_2 sites from rabbit kidney can be physically separated using affinity chromatography (Parini *et al.*, 1989).

The autoradiographic distribution of α_2-adrenoceptors and I_2 sites in rat brain, which has been dealt with in detail in Sections 2.2.1 and 3, is distinct. For example, the area postrema contains a low density of α_2-adrenoceptors and a very high density of I_2 sites, while in the ventral region of the anterior olfactory nucleus, the converse is true (Mallard *et al.*, 1992). This is illustrated graphically in Fig. 4. De Vos *et al.* (1991, 1994) have also demonstrated that α_2-adrenoceptors and I_2 sites are differentially distributed in human brain. This observation should be regarded with some caution, however, since measurement of the density of α_2-adrenoceptors and I_2 sites in postmortem human brain is compromised by the fact that the density of each is age-dependent (Cash *et al.*, 1987; Pascual *et al.*, 1992; Sastre and García-Sevilla, 1993).

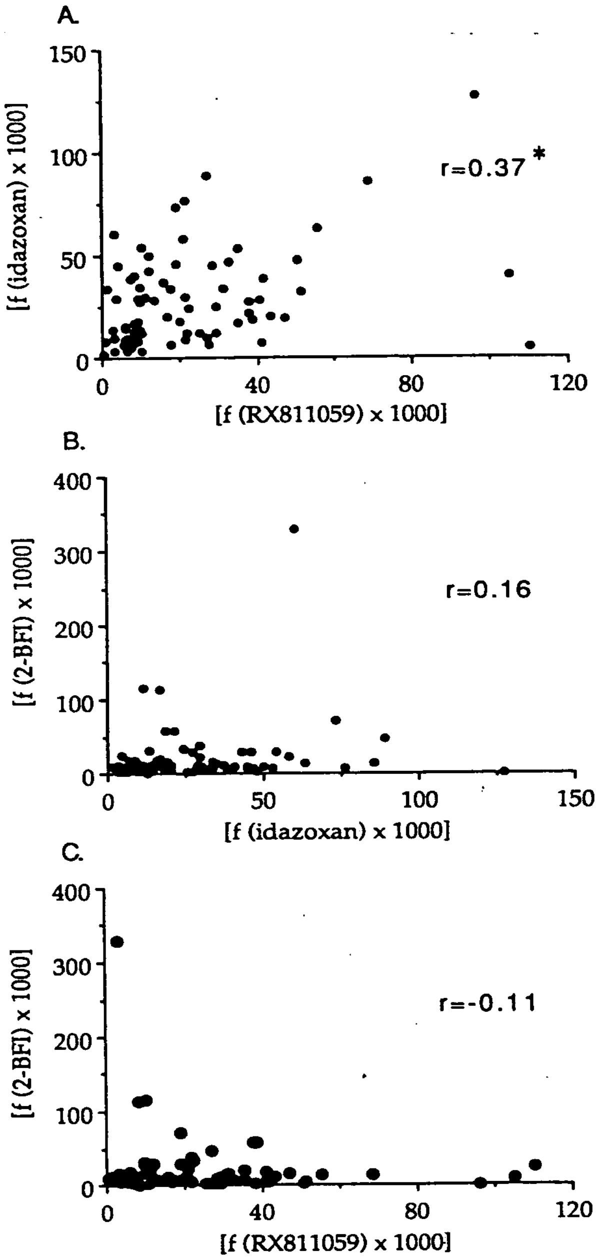


Fig. 3. Comparison of the effects of idazoxan, RX811059 and 2-BFI on local cerebral glucose use in the rat. Each data point represents the *f* value generated for a single brain region in response to (A) idazoxan and RX811059, (B) 2-BFI and idazoxan, and (C) 2-BFI and RX811059. The value of *f* was calculated according to the following formula: $f = \Sigma(x_C - x_{Ti})^2$, where x_C is the mean of $\log_e$(LCGU) for the control (saline-treated) group, and x_{Ti} is the mean of $\log_e$(LCGU) for the *i*th treatment group (see Ford *et al.*, 1985). Statistical significance was assessed by correlation analysis: $*P < 0.01$.

α_2-Adrenoceptors and I_2 sites are also differentially modulated by cations. Thus, [^{3}H]idazoxan binding to I_2 sites in a number of tissues is potently inhibited by K^+ ions, while Na^+ ions have little effect (Michel, M. C. *et al.*, 1989; Zonnenschein *et al.*, 1990; Lachaud-Pettiti *et al.*, 1991; Yablonski and Dausse, 1991). In contrast, α_2-adrenoceptor binding is highly sensitive to Na^+ ions, while the

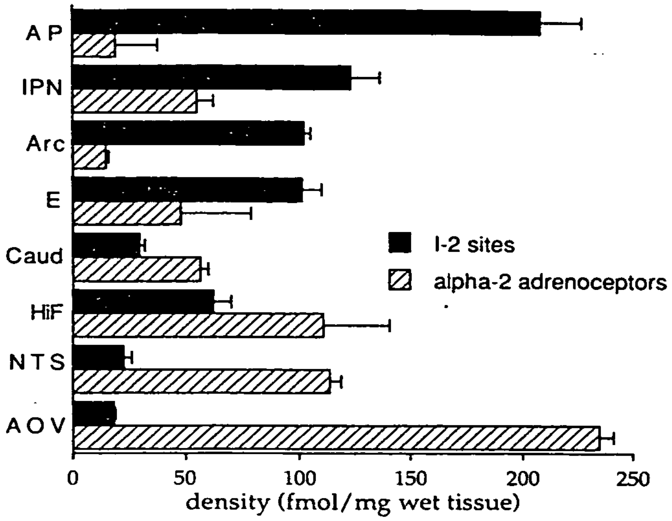


Fig. 4. Comparison of the distribution of I_2 sites and α_2-adrenoceptors in rat brain. Abbreviations: AOV, anterior olfactory nucleus (ventral); AP, area postrema; Arc, arcuate nucleus; Caud, caudate nucleus; E, ependyma; HiF, hippocampal fissure; IPN, interpeduncular nucleus; NTS, nucleus tractus solitarius. Data from Mallard *et al.* (1992).

effect of K^+ ions is much less pronounced (Greenberg *et al.*, 1978; Glossman and Presek, 1979; Coupry *et al.*, 1989; Yakubu *et al.*, 1990). Similarly, GTP alters the binding of agonists to α_2-adrenoceptors, but does not alter binding to I_2 sites (Glossman and Presek, 1979; Michel, M. C. *et al.*, 1989; Zonnenschein *et al.*, 1990; Lachaud-Pettiti *et al.*, 1991; Miralles *et al.*, 1993a).

4. SUMMARY AND CONCLUDING REMARKS

The presence of α_2-adrenoceptors in the mammalian CNS was first described almost two decades ago (Greenberg *et al.*, 1976). The subsequent development of highly selective ligands and the introduction of techniques such as radioligand-binding has greatly facilitated our understanding of this receptor population. For example, the distribution of α_2-adrenoceptors in the brain has now been described in some detail (Section 2.2.1). In addition, a number of functions, including blood pressure regulation, nociception, and the control of motor activity, are thought to be mediated by these receptors (Section 2.2.2). Accordingly, α_2-adrenoceptor agonists have several therapeutic applications, such as the treatment of hypertension and the management of perioperative, postoperative, and neurogenic pain, and their use as an adjunct to anaesthesia is well recognised (see Ruffolo, 1993, for a review).

More recently, it has been proposed that α_2-adrenoceptors may be classified into three or four subtypes (Section 2.1). This area of research is still very much in its infancy and awaits the development of subtype-selective compounds. Nevertheless, there is evidence to suggest that α_2-adrenoceptor subtypes are differentially distributed in the brain (Section 2.2.1), and some progress already has been made with regard to establishing whether the various functions assigned to α_2-adrenoceptors are mediated by distinct subtypes (Section 2.2.2). In terms of developing novel therapeutic agents with fewer side effects, the implications of this are obvious.

In comparison with the α_2-adrenoceptor, the I_2 site is a fairly novel concept. The presence of this site in the CNS is well established (Section 3), and its distribution here has been described (Section 3.3). Its function is less well defined, although there is evidence to suggest that it is involved in the regulation of feeding behaviour, MAO activity, and GFAP expression (Section 3.4). This translates to a possible use of I_2-selective ligands, a number of which have now been developed (Section 3.2), in the treatment of eating disorders, depression, and possibly also diseases characterised by neuronal loss. As they say, time will tell.

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